

X-Ray Microanalysis of Apples Treated with Kaolin Indicates Wax-Embedded Particulate in the Cuticle

Eric Curry
USDA, ARS
Tree Fruit Research Laboratory
Wenatchee, WA 98801
USA

Donald Baer and James Young
USDOE
Environmental Molecular Sciences Laboratory
Richland, WA 98801
USA

Keywords: *Malus × domestica*, kaolin, sunburn, heat injury, wax, micro-cracking

Abstract

'Gala' apples trees were treated with kaolin (Surround) in mid-July, and fruit were sampled 1 and 7 days after treatment. Apples were gently rinsed with cold deionized water to remove loose surface material. Small sections of cuticle tissue were shaved from the fruit surface with a razor blade and flash frozen on an aluminum block held at -190°C with liquid N₂. The tissue was freeze-dried, coated with a thin film of carbon, and examined using a field emission scanning electron microscope coupled with an energy dispersive x-ray microanalysis system capable of light element detection. Qualitative secondary electron imaging combined with quantitative elemental compositional analyses indicated kaolin particles may become embedded in the cuticle between individual wax platelets.

INTRODUCTION

Sun-induced injury (solar injury, SI) can be a costly problem in some years for tree fruit growers, especially in hot, arid regions. Although the injury to the fruit appears to be a combination of heat and solar radiation exposure (Lipton and O'Grady, 1980), synergistic thresholds for temperature, and radiation wavelength and intensity have not been clearly defined. To reduce this type of injury, therefore, either temperature or radiation, or both, must be reduced or regulated. Lipton and Matoba (1971) reduced the surface temperature of 'Crenshaw' melons (*Cucumis melo*, L.) by as much as 8 °C by applying a mineral whitewash material to the fruit surface, and thus reduced SI damage. Lipton (1972) also demonstrated that mineral whitewash applied to the melon fruit surface reduced internal tissue temperature and, as a result, improved fruit quality. (Mineral whitewash contains kaolinite, bentonite, attapulgite, as well as spreading and sticking agents). More recently, Glenn et al. (2002) reported kaolin alone reduced solar injury to apple fruit.

Kaolin, also known as kaolinite, is a clay mineral with the chemical composition Al₄(OH)₅O₂Si₂. The basic building blocks are layers of silica tetrahedra and layers of alumina octahedra in a 1:1 relationship. The unit cell repeats in the x and y direction to form extensive sheets in the xy plane, and in the z direction to form stacks of sheets (Barak and Nater, 2002). When placed in water the platelets shift apart, each plate attracting water to its negative face. Kaolin particulate may absorb 7 to 10 times its own weight, consequently swelling up to 18 times its dry volume.

Because of these characteristics, we wanted to know first, whether small particles would lodge in the micro-cracks of the fruit cuticle surface, second, whether the particles would become an integral part of the cuticle as the micro-cracks continued to 'heal' with new growth of wax platelet, and third, because of the marketability of appearance, whether the integration of clay particles within the cuticle matrix would alter the properties of the cuticle itself.

MATERIALS AND METHODS

Mature 'Gala'/EMLA MM.106 apples trees in northcentral Washington were treated with a single application of kaolin (Surround) in mid-July. Fruit were sampled 1 and 7 days after treatment, placed in a cooler held at 34°C and transported to the

laboratory in Wenatchee. Apples were gently rinsed with cold deionized water to remove loose surface material. Other than water, the area selected for examination was untouched. After drying briefly, a section of peel tissue about 0.3 mm thick and 5 mm in diameter was excised with a razor and flash frozen on an aluminum block held at -190°C . The tissue and cold aluminum block were transferred to a vacuum dessicator from which it was freeze-dried for 36 hours. Freeze-dried peel was fixed to a 12 mm aluminum stub and coated with a thin film of carbon. Samples were kept under slight vacuum and transported to the Environmental Molecular Sciences laboratory in Richland, WA for examination. Tissue was examined using a LEO 982 Field Emission Scanning Electron Microscope (FESEM, LEO Electron Microscopy Ltd. Cambridge, UK) equipped with an Oxford ISIS energy dispersive x-ray microanalysis system (Oxford Instruments, Oxford, UK) capable of light element detection.

RESULTS AND DISCUSSION

Apple fruit cuticle develops from wax platelets. During normal growth, when the fruit enlarges, the cuticle must also 'expand' resulting in "micro-cracks" on the surface (Fig. 1). Kaolin applied to the fruit during fruit enlargement lodges in these micro-cracks. The flake-like nature of kaolin is structurally suited to intercalation within the cuticle matrix (Fig. 2).

When apple peel was examined 1 day after treatment using scanning electron microscopy coupled with energy dispersive x-ray microanalysis, a probe of wax alone (identified in the primary electron image) resulted in a carbon (C) peak height of about 290 counts $\cdot\text{s}^{-1}$ and an oxygen (O) peak height of about 20 counts $\cdot\text{s}^{-1}$ (Fig. 3a). Based on the secondary electron image, we probed an area that appeared to be kaolin alone which resulted in a reduction in the height of the C peak (67 counts $\cdot\text{s}^{-1}$), and increases in the peak height of O (65 counts $\cdot\text{s}^{-1}$), aluminum (Al, 52 counts $\cdot\text{s}^{-1}$), and silicon (Si, 45 counts $\cdot\text{s}^{-1}$), and to a lesser extent, sodium (Na, 5 counts $\cdot\text{s}^{-1}$) and sulphur (S, 4 counts $\cdot\text{s}^{-1}$) (Fig. 3b).

Seven days after treatment, a second fruit was harvested and examined as described above. As in Fig. 3a & 3b, we could find areas appearing to be either wax alone or kaolin alone. In addition to these two individual compositional determinations, however, we were also able to locate areas which had characteristics of wax plus kaolin. When these areas were probed, the C peak jumped back up to about 200 counts $\cdot\text{s}^{-1}$ whereas the Al and Si peaks were reduced by about 50% (Fig. 4). These data suggest new wax platelets have covered the kaolin particulate within the expansion-induced cuticle micro-cracking, indicative of the crack-healing or 'curing' process.

CONCLUSIONS

The third phase of this study which was to investigate whether functional properties of the cuticle itself have been affected by the inclusion of kaolin particulate with the cuticle matrix is presently under way. Because the strength of the cuticle appears to develop as components of the wax plates polymerize, we could hypothesize a reduction in the shear strength of the cuticle polymer itself. Cuticle strength might be further reduced because the hydrophilic nature of the foreign particulate is in opposition to the hydrophobic nature of the wax-composed platelets. Other possible effects in addition to changes in cuticle shear strength might include changes in porosity, hydrophobicity, elasticity or even fruit nutritional properties. There may be a reduction in energy and substrate required for wax production if there is less void in the micro-cracks to fill. We have seen other micro-particles, presumably from blowing dust, also lodge within apple cuticle micro-cracks over which wax platelets are growing (Fig. 5). There may be changes in cuticle reflectivity or translucency, or changes in transmission of UV radiation which is necessary for proper pigmentation (colored cultivars). On the other hand, with regards to appearance, there may be no observable effects whatsoever. Nevertheless, because the incidence of cuticle related disorders is increasing (Curry, personal observation), more work is needed to determine if multiple applications of kaolin and incorporation of

particulate within cuticle matrices alter properties of fruit cuticles and, therefore, fruit quality or fruit storage potential beyond the obvious reduction in SI. We are currently examining these possibilities.

Literature Cited

- Barak, P. and Nater E.A. 2002. The Virtual Museum of Minerals and Molecules. <http://www.soils.wisc.edu/virtualmuseum>.
- Glenn, D.M., Prado, E., Erez, A., McFerson, J. and Puterka, G.J. 2002. A Reflective, processed-kaolin particle film affects fruit temperature, radiation reflection, and solar injury in apple. *J. Amer. Soc. Hort. Sci.* 127(2):188–193.
- Lipton, W. J. 1972. Temperatures and net heat gain in normal and whitewashed cantaloupe fruits. *J. Amer. Soc. Hort. Sci.* 97:242-244.
- Lipton, W.J. and O'Grady, J.J. 1980. Solar injury of 'Crenshaw' muskmelons: The influence of ultraviolet radiation and of high tissue temperatures. *Agr. Meteorol.* 22:235–247.
- Lipton, W.J. and Matoba, F. 1971. Whitewashing to prevent sunburn of 'Crenshaw' melons. *HortScience* 6:343–345.

Figures

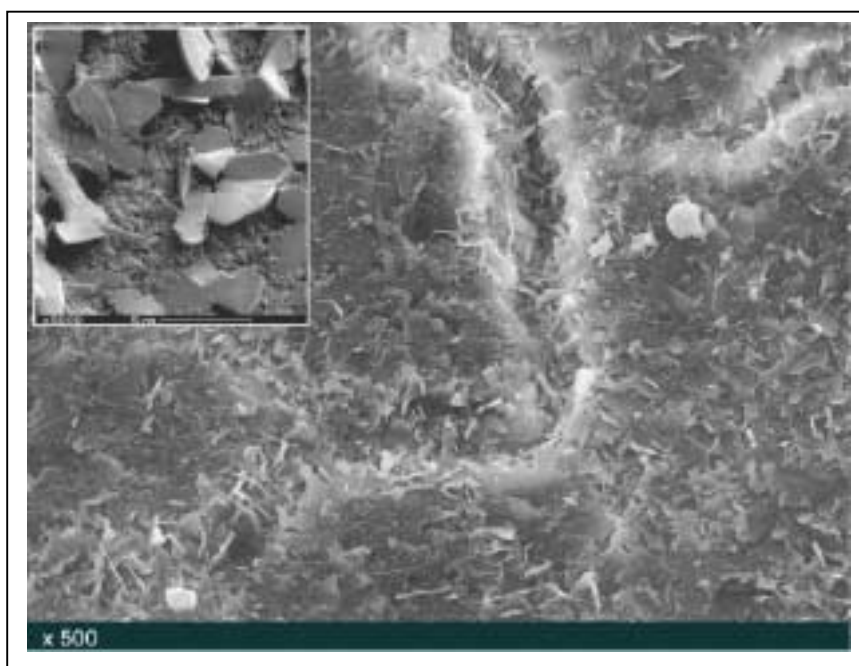


Fig. 1. Untreated apple peel. Micro-cracking of the cuticle is a natural result of fruit expansion. Wax platelets (inset) are the basis of cuticle development and continued growth. (Inset 10,000 X)

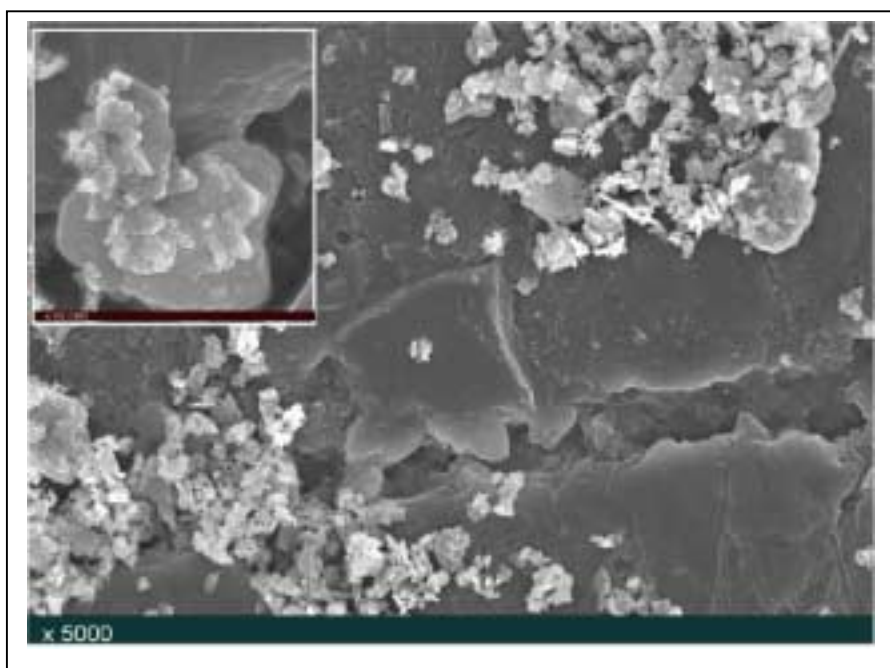


Fig. 2. Applied to the apple fruit surface, kaolin particles lodge in the cuticle microcracks. The flake-like nature of kaolin (inset) is structurally compatible to becoming sandwiched between wax plates. (Inset 10,000 X)

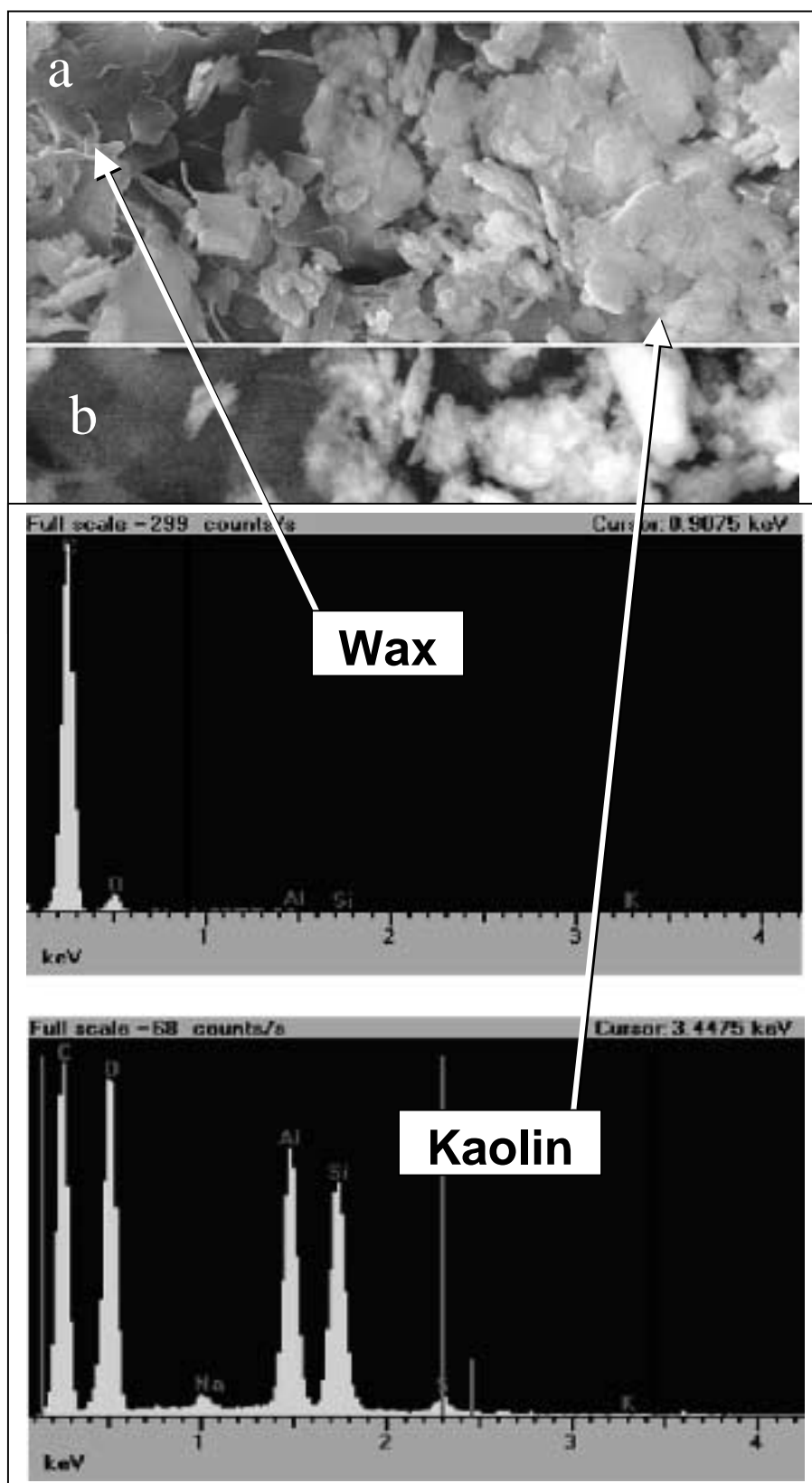


Fig. 3. Scanning electron micrograph of kaolin treated apple peel primary (a) and secondary (b) electron images. Arrow points to area probed for analysis. (see Fig. 4)

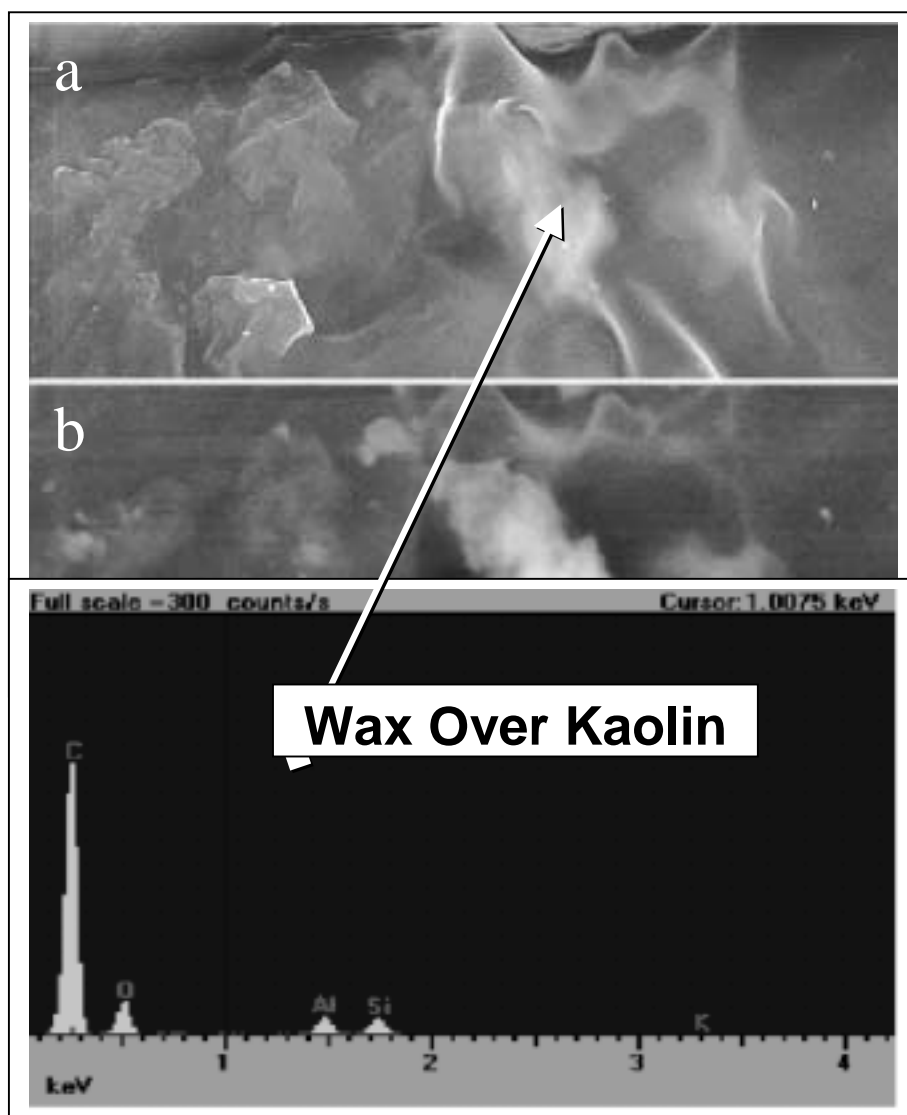


Fig. 4. Scanning electron micrograph of kaolin treated apple peel primary (a) and secondary (b) electron images, as well as the corresponding analysis of the region at which the arrow is pointed.

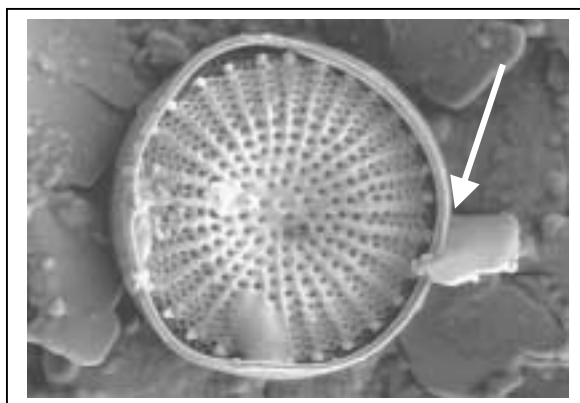


Fig. 5. Scanning electron micrograph (10,000 X) of diatom in micro-crack of 'Granny Smith' cuticle. Arrow points to wax platelet forming over object.